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Soil moisture effects on uptake of metals by *Thlaspi*, *Alyssum*, and *Berkheya*

J. Scott Angle[1,4], Alan J. M. Baker[2], Steven N. Whiting[2] & Rufus L. Chaney[3]
[1]*Department of Natural Resource Sciences and Landscape Architecture, University of Maryland, College Park, MD 20742, USA.* [2]*School of Botany, The University of Melbourne, Parkville, VIC 3010, Australia.* [3]*US Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705 USA.* [4]*Corresponding author**

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Abstract

Most commonly used hyperaccumulator plants for phytoextraction of metals evolved on soils where moisture is limited throughout much of the year. As these plant species are commercialized for use, they are frequently moved from the point of evolution to locations where environmental conditions may be significantly different. Greatest among these potential differences is soil moisture. The objective of this study was therefore to determine whether these plants could grow in soils with much higher soil moisture and whether they would continue to hyperaccumulate metals as soils approach saturation. We examined extractable soil metal concentrations, plant growth, and metal accumulation for the Ni hyperaccumulators, *Alyssum murale* and *Berkheya coddii* and the Zn hyperaccumulators *Thlaspi caerulescens* cultivars AB300 and AB336. Non-hyperaccumulating control species for each were also examined. In general, extractable soil concentrations of Ni decreased with increasing soil moisture content. Few significant effects related to Zn extractability were observed for any of the soil moisture treatments. The biomass of all tested species was generally greater at higher soil moisture and inhibited at low soil moisture. Further, plants accumulated large amounts of metals from soil at higher soil moisture. Highest foliar concentrations of Zn or Ni were found at the two highest WHCs of 80 and 100%. These results show that hyperaccumulators grow well under conditions of high soil moisture content and that they continue to hyperaccumulate metals. Thus, growing *Thlaspi, Alyssum*, and *Berkheya* for commercial phytoextraction under nonnative conditions is appropriate and suggests that this technology may be applied to a wide and diverse range of soil types, climatic conditions, and irrigation regimes.

Abbreviations: WHC – water holding capacity

Introduction

Phytoextraction of heavy metals from soil was first proposed over 20 years ago and has since found mainstream acceptance within the scientific and regulatory community (Baker et al., 1991; Chaney, 1983). Since that time, the technology has matured with the publication of many reports detailing both basic and applied aspects of phytoextraction (Lasat, 2002). Despite all of the work reported to date, many fundamental questions remain to be answered if plant growth and metal uptake are to be maximized.

Most of the hyperaccumulators used for experimental or commercial purposes were originally collected in arid regions of the world, or where soil water holding capacity is low. Species in the genus *Alyssum* are the most important hyperaccumulator of nickel and cobalt and originate from arid Mediterranean regions around Turkey and Greece (Baker and Brooks, 1989, Reeves, 1992). This area of the world is subject to extreme summer drought along with high daytime temperatures.

The most important hyperaccumulators for zinc and cadmium are in the genus *Thlaspi*. Many hyperaccumulators of *Thlaspi* were originally collected on skeletal, shallow soils with very low water hold-

* FAX No: +1-301-314-9089. E-mail: ja35@umail.umd.edu

ing capacity (Reeves and Brooks, 1983a,b). Although no work has examined the ability to survive drought in soil culture, conditions under which they evolved suggest that adaptation to drought prone soils is an important characteristic of these species.

Most metals, including Zn and Ni are generally less available at high soil moisture due to the effect of reducing conditions on the metal ion. In waterlogged soils, most metals, including Zn, Cd, and Ni exhibit complicated solubility with generally reduced solubility due to low redox potential (Rieuwerts et al., 1998) and formation of sparingly soluble sulfides (Marschner, 1995). Hammer and Keller (2002) have recently shown that the hyperaccumulator *Thlaspi* differentially accesses various pools of metals within soil. Further, plant growth, exclusive of metal availability may be affected when hyperaccumulators are grown in high moisture soils.

High metal bioavailability is likely to be important for hyperaccumulators. Li et al (1997) showed that the activity of Zn required for 'normal growth' of *T. caerulescens* is 10^4-fold higher than non-accumulator plants. When *T. caerulescens* is grown at low or 'normal' Zn concentrations, the plants grow poorly and exhibit a variety of nutrient imbalances. Further, high metal uptake appears to be important in the protection of plants from fungal attack. When *A. murale* and *A. serpyllifolium ssp. lusitanicum* are grown in nutrient solution with low Ni concentrations, plants are more susceptible to infection with *Pythium ultimum* (Ghaderian et al., 2000). *Alyssum* grown with higher Ni concentrations were protected from fungal attack.

Despite the obvious importance of soil moisture on metal uptake by hyperaccumulators, we are not aware of any studies that have examined this interaction. A study was therefore conducted where soil moisture regimes were established and metal uptake by hyperaccumulating and nonaccumulating plants was observed. The study was designed to describe only changes in metal uptake since many incidental factors affected by soil moisture can influence this interaction. Disease incidence and macronutrient availability, for example, can all affect plant growth and thus metal uptake.

Materials and methods

Soils

Two soils were collected for testing of soil moisture effects on metal uptake. The first soil is a serpentine (Ni, Co and Cr rich) soil collected from the base of Mt. Wellington, Victoria, Australia, and has been previously described by Chesterfield (1978). Soil was collected from the top 10 cm of the soil profile.

The soil is a clay loam with dark reddish brown color. It has a pH of 6.0, an organic matter content of 2.5% and a water holding capacity of 80.7%. Total metals were determined after extraction with concentrated HCl/HNO_3 (McGrath and Cunliffe, 1985). The soil contained on a dry weight basis concentrations of Ni, Ca, Co, Mg, Mn and Zn of 1,192, 590, 67, 24, 461, 1,422 and 62 μg g^{-1}, respectively.

A second soil was collected from an agricultural field approximately 500 km outside Melbourne, AU. The soil is classified as a Wagga Wagga soil and contained very low concentrations of both Zn and Ni. The soil pH was 6.5 and the organic matter content was approximately 1.5%. The water holding capacity of this soil was at 45.2% moisture on a dry weight basis.

Both soils were air dried and sieved through a course mesh screen (4 mm) to remove rocks and other large organic materials. Soils were held at room temperature until use.

To determine soil water holding capacity (WHC), triplicate soils were placed into cores with a mesh net bottom. They were saturated with distilled water and allowed to drain for 18 h while the top was sealed to prevent evaporation. At the end of the period, the soil in the cores was weighed and recorded. Soils were then dried overnight at 80 °C for 18 h followed by drying at 104 °C for 2 h and reweighed. The soil moisture content prior to drying was defined at 100% WHC or field capacity. The Wagga Wagga soil was next split and a portion of the soil amended with $NiCl_2{\cdot}6H_2O$ to a rate of 500 μg Ni g^{-1}. Nickel was added according to Kabata-Pendias and Pendias (2001) to be toxic to most plants. Nickel chloride was added by first dissolving the metal salt in distilled water then adding the appropriate amount of the stock solution to the soil. Soils were thoroughly mixed and allowed to incubate 1 month prior to sowing. Following initial incubation, all pots were flushed with several pore volumes of distilled water in order to leach any excess salts that developed during incubation.

The serpentine soil was not amended with additional Ni, since it already contained a high Ni content. However, the soils were treated identically to the soil above.

Zinc sulfate ($ZnSO_4 \cdot 7H_2O$) was added to the Wagga Wagga soil only to achieve Zn toxicity to non-

hyperaccumulator plants but which would have no adverse effect on Zn hyperaccumulators. Zinc sulfate was added to soil to achieve a final soil concentration of 1000 μg Zn g^{-1}. We used $ZnSO_4 \cdot 7H_2O$ because Whiting et al. (2001b) had previously shown that Zn was highly available from this salt and was readily taken up by *T. caerulescens*.

Since SO_4 was expected to reduce soil pH, we added a control treatment where an equilmolar amount of H_2SO_4 was added to soil. It was first mixed with a small amount water that was then thoroughly incorporated into the soil. As before, H_2SO_4 was added dissolved in water and the soil was well mixed and leached prior to use.

Prior to adding soils to pots, they were amended with NH_4NO_3 to supply 200 kg N per ha. Potassium phosphate was also added to supply 100 kg P per ha since the serpentine soil, in particular, is known to be deficient in P. Amendments were thoroughly mixed into the soil. Soils were then added to 10-cm pots containing 500 g soil each.

Soils were next amended with distilled water to achieve soil WHC of 30, 40, 60, 80, and 100%. A WHC of 20% was previously shown to be too low to support growth of *Thlaspi* or *Alyssum*. Water was added every other day by weight throughout the duration of the study.

Plants

Immediately after establishment of soil moisture treatments, soils were sown with appropriate plants species and genotypes. All plants had been pregerminated in the soil into which they were to be later transplanted. Plants were approximately three weeks old when transplanted into treatment soils.

For the Ni-rich and control soils, the Ni hyperaccumulator *Alyssum murale* (AB00075f) from Panorama, Thessaloniki Greece and *Berkheya coddii* (AB00321f) from South Africa were selected. Both species have been extensively studied and we have been working to develop these plants for commercial use. Both species have been reported to hyperaccumulate Ni in excess of 2% (Baker et al., 2000).

There is no completely appropriate control plant species for comparison with *B. coddii* and *A. murale*. Most researchers requiring a control have used *A. montanum* cv. 'Mountain Gold'. While the same genus as *A. murale*, this species is quite different in that it is a true annual, while *A. murale* is a perennial. Many other grow habits are also dissimilar, yet this remains the most widely used control for comparison. We therefore used this species knowing of its many limitations as a control.

For a Zn hyperaccumulator, we selected *Thlaspi caerulescens* since this is the best studied of all the Zn accumulators (Baker and Whiting, 2002; Brown et al., 1994, 1995; Lasat, 2002). Further, *T. caerulescens* is known to be quite variable in both growth habit and Zn accumulation. This is most likely due to the extreme variability of the European locations from which these plants were collected (Reeves and Brooks, 1983a, b). For this reason we studied two different genotypes of *T. caerulescens* AB300 and AB336. *Thlaspi caerulescens* AB 300 was originally collected from Whitesike Mine in Cumbria, UK while *T. caerulescens* AB 336 was collected in Prayon, Belgium. As a non-hyperaccumulating control we used *Thlaspi arvense*. While this species does not accumulate Zn, it has many of the limitations noted above for *Alyssum.* Again, there is no perfect control for *T. caerulescens.*

Analytical procedures

Initially three plants were transplanted into each pot to ensure at least one would survive the transplant, but were thinned to one plant per pot within 10 days. Pots were placed into a greenhouse (averages; 24 °C, 40–50% RH, 150 mol m^{-2} s^{-1} PAR) where they were maintained for a period of 3 months and watered as needed.

At the end of this time, plants were harvested by cutting at the crown area. Shoots were washed to remove adhering soil particles in a distilled water mix containing several drops of the surfactant Tween 80. Next they were rinsed in distilled water, placed in paper bags and dried at 80 °C for 12 h. Shoot dry weights were recorded.

Shoot tissue was ground in a stainless steel Wiley Mill and subsamples digested in concentrated HNO_3 at 90 °C for 2 h and diluted to 10 mL. The metal concentration of the digest solutions was determined on an atomic absorption spectrophotometer with background correction. Appropriate blanks and standard tissue samples were run to assure analytical integrity (Whiting et al., 2001a).

Soil samples collected from the pots at the end of the experiment were extracted with 10 m*M* $Sr(NO_3)_2$. Briefly, 10 g soil was shaken in 50 mL $Sr(NO_3)_2$ for 2 h and at this time, the suspension was filtered through Whatman #40 filter paper. Samples were stored at 4 °C after the addition of a single drop of conc. HCl to pre-

vent bacterial growth. Extracts were run on an atomic absorption spectrophotometer as before.

Each of the soil, plant, and soil moisture treatments were run in triplicate. In addition, for each of the metal determinations, analyses were run in duplicate. All data were analyzed using SAS version 6.12 (SAS Institute, Cary NC). An analysis of variance was conducted and where significant treatment differences were detected, means were separated according to the Duncan's multiple range Test at the 5% level of significance.

Results

Nickel

Strontium nitrate (10 m*M*) was used for extraction of metals from soil. Although there is no universal extractant for all plant species and soils, a strontium nitrate extractant has been shown to provide reasonable estimates to Ni uptake into hyperaccumulator plants.

Higher extractable Ni concentrations were found in soil amended with the $NiCl_2$ salt as might be expected since it was unlikely that Ni was fully in equilibrium with the soil matrix over such a short period of time (Table 1). Extractable Ni generally declined with increasing soil moisture content, although this was most pronounced in the serpentine soil where lowest extractable concentrations were consistently found at the highest soil moisture content. The difficulty in making generalized comments related to soil moisture is that both plant growth and Ni uptake complicate analysis and interpretation. For example, at highest soil moisture content, plants often grew better and thus removed greater quantities of Ni from soil. There were no significant effects of soil moisture content on the extractable metal concentrations in the unamended, control soil.

Dry weight of *A. murale*, *B. coddii* and *A. montanum* was always lowest at the two lowest soil moisture contents (Table 2). Above 60% WHC there was no particular trend in shoot mass related to soil moisture. Some plants grew best at 100% WHC while others grew best at 60% WHC. In the unamended, control soil without the added burden of Ni toxicity or nutrient deficiency, all species grew best at 100% WHC.

Alyssum murale grew best on the $NiCl_2$ amended soil, especially at the higher soil moisture contents (Table 2). This species grew quite poorly on the unamended soil reflecting it's higher Ni requirement. *Berkheya coddii* generally grew best on the $NiCl_2$ amended soil and the unamended soil. This was most likely due to the low fertility of the serpentine soil. Serpentine soils are notoriously low in a number of important plant nutrients including calcium and phosphorus while having a higher Mn concentration. *Alyssum montanum* grew very poorly on the serpentine soil that was most likely due to the combined effects of low soil fertility, Ni toxicity and potentially Mn toxicity (although this was not measured). At low soil WHC, *A. montanum* grew best on the Ni salt amended soil, but at higher soil moisture, it grew best on the unamended control.

Foliar Ni concentration integrates both plant growth and Ni availability in soil. *Berkheya coddii* generally had the highest foliar Ni concentration for all soil moisture values for soils with significant available Ni (Table 3). The only exception was at 100% WHC for all three soils, where *A. murale* exhibited a higher foliar Ni concentration compared to *B. coddii*. Highest foliar Ni concentration (for all species) was usually found at either 80 or 100% WHC. The highest foliar Ni concentration, 2.07%, was observed for *B. coddii* in the serpentine soil at a WHC of 80%. Nickel uptake by *A. montanum* was consistently low with the highest observed concentration of 50 μg g^{-1}. In the unamended, control soil, *A. murale* contained much higher concentrations of Ni than either *B. coddii* or *A. montanum.* This demonstrates the extreme ability of this species to scavenge Ni from soils even with low Ni concentration.

Zinc

Effects of soil moisture on Zn solubility are presented in Table 4. Low extractable Zn was found for all soils amended with sulfate only since no Zn was added to any of the soil moisture treatments. There were no significant differences in Zn solubility for any of the sulfate amended soil moisture treatments.

Zinc solubility was confounded by a corresponding reduction in pH associated with the addition of $ZnSO_4$. The SO_4 ion will reduce soil pH and is well known to increase Zn solubility in soil. Kayser et al. (2001) added elemental S to soil and measured both extractable Zn and Cd from soil as well as uptake by several metal tolerant, but non hyperaccumulator plants. These authors found that as soil pH was reduced to 4.0 during oxidation of the elemental S, both extractability and

Table 1. Effect of soil moisture content on Ni solubility in soil

WHC[1] (%)	Species								
	Ni^+ (μg kg^{-1})			Serpentine (μg kg^{-1})			Ni^- (μg kg^{-1})		
	A. murale	*B. coddii*	*A. montanum*	*A. murale*	*B. coddii*	*A. montanum*	*A. murale*	*B. coddii*	*A. montanum*
30	1026.0 a[2]	501.6 ab	1700.5 a	813.0 a	576.3 a	573.7 a	2.2 a	39.7 a	23.3 a
40	1046.3 a	572.3 a	1324.7 a	628.5 ab	683.0 a	587.7 a	64.3 a	40.3 a	51.7 a
60	578.3 b	373.6 b	1094.3 a	588.9 b	539.0 a	679.0 a	0 a	0 a	69.0 a
80	875.7 ab	589.0 a	1047.3 a	496.3 b	650.3 a	592.3 a	11.5 a	0 a	11.0 a
100	975.0 ab	475.3 ab	864.5 a	485.6 b	469.7 a	477.7 a	1.6 a	68.3 a	23.0 a

[1]WHC – water holding capacity. [2]Means followed by the same letter are not significantly different at $P<0.05$ within the same column.

Table 2. Effect of soil moisture content on plant dry weight

WHC[1] (%)	Species								
	Ni^+ (mg)			Serpentine (mg)			Ni^- (mg)		
	A. murale	*B. coddii*	*A. montanum*	*A. murale*	*B. coddii*	*A. montanum*	*A. murale*	*B. coddii*	*A. montanum*
30	26.8 c[2]	53.0 c	3.7 c	27.4 b	10.7 b	1.4 a	3.3 b	114.7 a	1.4 b
40	108.8 c	45.4 c	28.3 bc	32.7 b	49.6 b	4.7 a	53.8 b	43.6 a	18.5 b
60	306.9 b	105.0 b	215.3 a	132.1 a	52.0 b	9.9 a	52.8 b	119.7 a	207.6 a
80	472.3 a	158.3 a	115.9 abc	78.4 ab	260.6 a	3.1 a	185.0 a	95.9 a	256.2 a
100	266.5 b	80.6 bc	135.8 ab	115.6 a	53.2 b	1.7 a	220.1 a	154.2 a	373.9 a

[1]WHC–water holding capacity. [2]Means followed by the same letter are not significantly different at $P<0.05$ within the same column.

Table 3. Effect of soil moisture content on plant Ni concentration

WHC[1] (%)	Species								
	Ni^+ (mg kg^{-1})			Serpentine (mg kg^{-1})			Ni^- (mg kg^{-1})		
	A. murale	*B. coddii*	*A. montanum*	*A. murale*	*B. coddii*	*A. montanum*	*A. murale*	*B. coddii*	*A. montanum*
30	5032 b[2]	11781 a	23 a	7917 a	6475 b	0 a	84.5 b	5.5 b	0 a
40	5863 b	10059 a	42 a	8858 a	9634 b	33 a	197 ab	13 ab	6 a
60	9833 a	4542 b	40 a	5389 a	9102 b	32 a	337 a	38 a	3 a
80	10789 a	12189 a	48 a	9054 a	20725 a	27 a	290 a	63 a	7 a
100	11035 a	9976 a	50 a	13161 a	9908 b	0 a	220 ab	31 a	6 a

[1]WHC–water holding capacity. [2]Means followed by the same letter are not significantly different at $P<0.05$ within the same column.

Table 4. Effect of soil moisture content on Zn solubility in soil

WHC[1] (%)	Species					
	Zn (μg kg^{-1})			SO_4 (μg kg^{-1})		
	T. caerulescens		*T. arvense*	*T. caerulescens*		*T. arvense*
	300	336		300	336	
30	338.7 c[2]	487.3 a	1013.0 a	76.0 a	63.7 a	64.0 a
40	917.5 b	910.7 a	532.7 bc	20.0 a	78.0 a	92.0 a
60	779.7 b	666.7 a	507.1 c	30.0 a	50.3 a	117.0 a
80	510.3 c	919.7 a	428.0 c	60.7 a	63.0 a	39.7 a
100	1270.3 a	848.0 a	867.3 ab	103.0 a	23.0 a	73.7 a

[1]WHC–water holding capacity. [2]Means followed by the same letter are not significantly different at $P<0.05$ within the same column.

plant uptake of Zn were markedly increased. For comparison, we included a H_2SO_4 treatment in addition to the $ZnSO_4$ to help identify effects due to the metal, and effects due simply to a reduction in soil pH

Where Zn was added to soil, extractable concentrations were all higher than the sulfate amended soil. For *T. arvense*, the highest extractable concentration was at 30% WHC. This may have partially resulted from the fact that *T. arvense* is a nonaccumultor, plus plant growth was poor at this WHC and thus little Zn was taken up into the plant. For *T. caerulescens* AB300 and AB336, the highest Zn solubilities were observed at 100 and 80% WHC, respectively. Lowest Zn solubilities were seen at 30% WHC.

Growth of *T. caerulescens* AB300 was highest at a WHC of 100% and lowest at a WHC of 30% (Table 5). Genotype AB336 responded somewhat differently with highest and lowest growth observed at 60/80 and 100% WHC, respectively. Clearly this genotype was more sensitive to wet soils than genotype AB300. The non accumulator, *T. arvense* also showed highest growth at a WHC of 60%.

Few differences were observed when only sulfate was added to soil. This was due to the high level of variability in growth associated with AB300 and AB336. Growth of *T. arvense* was much higher with added sulfate, especially at the higher soil moisture levels. This demonstrates the Zn sensitivity of this species.

Foliar Zn concentrations were determined where there was adequate biomass for analysis. Generally at the lowest soil moisture content, growth was so low that there was not enough biomass for analyses (Table 6). In general, uptake of Zn was low compared to previously published reports using similar genotypes (Brown et al., 1994, 1995). The highest foliar Zn concentration observed was 6686 μg g^{-1}. Zinc uptake by *T. arvense* was higher from the Zn amended soil compared to the sulfate amended soil. This shows that even though *T. arvense* is not a true hyperaccumulator, it has the ability to take up more Zn compared to other non accumulating species.

There were few significant differences in uptake of Zn by any of the genotypes/species, soils, or soil moisture content (Table 6). When AB 300 and AB 336, however, were combined over all treatments, the highest Ni concentration was found at a WHC of 60%.

Discussion

Hyperaccumulating species of the genus *Thlaspi* are typically found to grow on skeletal soils that often have a very low water holding capacity. Although rainfall is generally high in areas where the seeds were collected for these experiments, the soil's water holding capacity is low, thus, they in effect evolved under transient low moisture conditions (Baker et al., 2000). Nearly all *Alyssum* used for phytoextraction evolved under Mediterranean conditions where summers are very hot and dry. Many of the soils where *Alyssum* was collected are also rocky, stony or sandy, with little capacity to hold soil moisture (Reeves, 1992). *Berkheya* evolved on drought-prone soils of South Africa. Thus, all species are likely to be adapted to tolerate conditions of drought and low soil moisture (Whiting et al., 2002).

Since these plants will be cultivated under conditions vastly different from the conditions under which they evolved, it is important to know whether plants will be able to grow and hyperaccumulate metals under varied soil moisture conditions. The environments where *Thlaspi* and *Alyssum* are most likely to be grown for phytoremediation and phytomining are nonskeletal soils with higher water holding capacity and thus higher soil moisture content. It is therefore important to understand how these plants will respond to cultivation under conditions other than which they evolved, especially wet soils. The primary objective of this study was therefore to examine the potential for hyperaccumulation under soils of varying soil moisture.

It is clear that *Thlaspi, Alyssum*, and *Berkheya* are able to grow well on soils with high soil moisture content. Further growth was not limited except at possibly the highest soil moisture content of 100% WHC. However, a WHC of 100% occurs only rarely and for generally brief periods of time, since soils typically drain quickly following rainfall or irrigation events.

This study supports the work of Whiting et al. (2002) who examined metal accumulation under various water stress regimes as mediated by aqueous polyethylene glycol. Whiting et al. showed that the process of metal hyperaccumulation was not associated with drought tolerance as was previously suggested in several other studies (Baker and Walker, 1989; Severne, 1974), and that the process of hyperaccumulation more likely related to protection from pathogens and herbivores. The current study, conducted in soil containing various soil moisture contents did not find any

Table 5. Effect of soil moisture content on plant dry weight

WHC[1] (%)	Species					
	Zn (mg)			SO_4 (mg)		
	T. caerulescens		*T. arvense*	*T. caerulescens*		*T. arvense*
	300	336		300	336	
30	2.2 b[2]	7.3 bc	7.3 b	6.0 a	7.7 a	4.6 c
40	6.7 b	8.9 bc	64.0 ab	5.8 a	6.9 a	132.4 bc
60	32.9 b	21.6 ab	96.9 a	42.5 a	12.6 a	135.6 bc
80	54.6 ab	24.4 a	15.0 b	52.6 a	8.5 a	482.8 a
100	111.6 a	4.2 c	22.0 b	9.0 a	5.2 a	299.4 ab

[1]WHC–water holding capacity. [2]Means followed by the same letter are not significantly different at $P<0.05$ within the same column.

Table 6. Effect of soil moisture content on plant Zn concentration

WHC[1] (%)	Species					
	Zn (mg kg^{-1})			SO_4 (mg kg^{-1})		
	T. caerulescens		*T. arvense*	*T. caerulescens*		*T. arvense*
	300	336		300	336	
30	ND[2]	ND	ND	ND	ND	ND
40	ND	1908 a	222 a	ND	194 a	39 a
60	6686 a[3]	3896 a	553 a	90 a	286 a	39 a
80	2762 b	2469 a	357 a	77 a	216 a	25 a
100	2253 b	2169 a	260 a	114 a	219 a	36 a

[1]WHC–water holding capacity. [2]ND: not determined. [3]Means followed by the same letter are not significantly different at $P<0.05$ within the same column.

link between low soil moisture and enhanced uptake of metals. In fact, just the opposite was observed where greater metal uptake was generally observed at higher soil moisture values. In addition, plants produce greater biomass at higher soil moisture levels that further enhances the amount of metals extracted from soil.

Growth and metal uptake at soil moisture contents higher than under which the plants evolved is an important attribute necessary for successful phytoremediation. Not only will soil moisture conditions likely be higher when grown away from the site of evolution, but there is also the potential for irrigation of the crop during times when rainfall is lacking. Based upon results of modeling approaches to determining metal uptake by *Thlaspi caerulescens*, agronomic management of the substrate, including irrigation and soil textural improvement, is likely to play an integral part of any phytoextraction practice (Robinson et al., 2003; Whiting et al., 2003). Indeed, the economics of phytoremediation are such that any growth stimulus resulting from irrigation will be cost effective (Angle et al., 2001).

One word of caution when considering water relationships with growth and uptake. We have occasionally observed in field situations that *Alyssum* spp. are rapidly killed from either root or flower blight diseases when soil moisture is very high and soil Ni concentration is low. It has been hypothesized that many hyperaccumulators have reduced their 'normal' organic based abilities to protect themselves against attack of pathogens and instead rely on metal toxicity as a means of protection (Davis and Boyd, 2000). When hyperaccumulators are grown in soil with low metal content, foliar metal concentration is low and thus they might be left defenseless.

We carefully monitored the current study for disease, especially at low metal concentration and high soil moisture, but except for increased incidence of powdery mildew on some plants at these treatments, no overt death or reduction in growth was observed. Further research on hyperaccumulators is needed to determine the exact role of disease at high soil moisture and low metal content.

In conclusion, this study has shown that *Thlaspi, Alyssum* and *Berkheya* grow well at high soil moisture

content, despite the fact that they generally evolved on soils with low moisture content, whether this was due to inherent low soil moisture holding capacity or low rainfall in the part of the world in which they evolved. In addition, where soil moisture content is low, plants showed a positive response to increasing soil moisture content. Both growth and metal uptake were generally increased with increasing soil moisture (at least up to a WHC of 80%). Since irrigation is necessary to maintain growth during much of the year and especially during the hot and dry months of summer, managers of phytoremediation and phytomining projects can be confident that growth will be positively affected along with a positive increase in metal uptake and thus phytoextration efficiency.

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